The Section 112, first paragraph, rejection of claims 25-41 and 45-50 will be moot upon entry of the above. The claims are submitted to be supported by an enabling disclosure and consideration of the following in this regard is requested.

The present invention relates to a composition comprising: a hypoxia and/or ischaemic and/or stress regulatable agent; a mononuclear phagocyte that has intemalised therein, the hypoxia and/or ischaemia and/or stress regulatable agent; and a binding agent that binds to a cell surface element of the mononuclear phagocyte wherein said binding agent is a viral vector and wherein said binding agent comprises means for ensuring the hypoxia and/or ischaemic and/or stress regulatable agent is intemalised into the mononuclear phagocyte.

The binding agent may be an adenoviral vector and the hypoxia and/or ischaemic and/or stress regulatable agent may comprise a therapeutic gene. It is well known in the art that genes may be transferred into non-dividing cells such as monocytes/macrophages using a replication defective adenoviral vector. By way of example, efficient transfer of genes into human macrophages has been achieved using a replication defective adenoviral vector with expression of the gene in 40-80% of the cells exposed to the vector and lasting up to 3 weeks after gene transfer (see page 36, lines 17-20). In addition, transfected monocytes/macrophages have been injected directly into disease tissue of a donor (see page 38, lines *3-5).*

Thus the applicants submit that detailed guidance for the preparation of

aspects of the specification.

The applicants provide the following additional data which clearly demonstrate:

(i) transduction of macrophages with adenoviral vectors comprising either a hypoxic regulatable (HRE) reporter gene (such as Lac Z) or CMV regulated reporter genes (such as GFP);

(ii) peritumoural/intratumoural injection of macrophages comprising either HRE regulated reporter gene (such as Lac Z) or CMV regulated reporter genes (such as GFP) into a murine xenograft model and intraperitoneal ovarian cancer model; and

(iii) hypoxic regulated expression of the reporter gene (such as LacZ) in the tumour site.

By way of explanation, the following data is provided to demonstrate intra- and peri-tumoural administration of adenovirally transduced macrophages into established MDA 231 tumour xenografts.

Example I

Macrophage preparation

Macrophage culture and transduction was carried out as described Griffiths *et al* 2000 (Gene Therapy, 7, 255-262, a copy of which is attached).

Adenoviral Vector Preparation

The adenoviral vector preparations used were an adenoviral vector comprising a CMV promoter and a green fluorescent protein reporter gene (Ad CMV GFP) and an [illegible] HRE and a LacZ reporter

and 66 p.f.u. respectively.

Adenoviral transduction of macrophages

The adenoviral vector transduction efficiency was routinely >80% as monitored by UV microscopy in Ad CMV GFP transduced cells.

MDA 231 tumour xenograft preparation in mice

Two 0.5cm diameter MDA 231 subcutaneous tumours were established on nu/**nu** mice.

Transfer of adenoviral transduced macrophages into mice with MDA 231 tumour xenografts

Approx $2x10^6$ macrophages ($1x10^6$ per tumour) were administered peritumourally or intratumourally in a volume of 100 μl per tumour. After three days the tumours were removed and immersed in formalin. The samples were paraffin wax embedded, sectioned and immunostained for GFP protein. Samples injected with untransduced macrophages were used as a negative control.

Peritumoural Administration

Peritumoural injections were given under the skin around the periphery of the tumour and great care was taken to avoid the needle penetrating the tumour mass.

Results I - *Peritumoural* Administration

Figure 1 shows strong positive staining for GFP in cells with macrophage morphology in the tumour xenograft injected with macrophages transduced with Ad.

untransduced macrophages and immunostained for Lac Z (control).

Figure 2(b) shows in the right hand column, sections of tumours injected with Ad.HRE. LacZ macrophages and immunostained for LacZ.

It is clear from a comparison of Figure 2A (x60) (control) with Figure 2 (B) (x60) (test) that there is evidence for switch on of the HRE promoter in macrophages transduced with Ad. HRE LacZ in the tumour xenograft as positive staining for the LacZ reporter gene is observed. It is also clear from Fig 2(B) (x60) that the macrophages are located in a necrotic region where an hypoxic environment is highly likely (brown indicates positive staining for macrophages).

Example 2

Intraperitoneal Studies

Macrophage preparation

As indicated above, macrophage culture and transduction was carried out as described in Griffiths *et al.* 2000 (a copy of which is attached).

Human intra-peritoneal ovarian cancer models

Three human intra-peritoneal ovarian cancer models were used. These were entitled IGROV, Ali and HU.

Ali and HU models were maintained by *in vivo* passage of human tumour cells in nude mice. IGROV tumours were generated from cell implants. Two weeks after passage the animals had established intraperitoneal tumour masses.

Transfer of adenoviral transduced macrophages into mice with ovarian

Macrophages transduced with Adenoviral CMV LacZ CMV GFP vector were injected in a volume of 200 μl intraperitoneally. Three days later the tumours, hearts livers, spleen and lungs were removed, snap frozen, cut and mounted using a cryostat and stained for LacZ expression by X-Gal histochemistry.

Results 2

Figure 3 illustrates the staining results obtained for IGROV, HUA and Ali tumour xenografts after intra-peritoneal administration of macrophages. The blue precipitate indicates positive staining for Lac Z. Co-localisation with both GFP and CD68 (which is a macrophage marker) immunohistochemistry was evident.

Summary

The above described results and attached clearly demonstrate that:

(i) an adenoviral transduced macrophage can deliver a nucleotide sequence of interest (NOI) to a target site; and

(ii) the NOI delivered by adenoviral transduced macrophage can be selectively expressed at a target site such as a hypoxic site when the expression of the NOI is regulated by a hypoxic response element (HRE).

The additional data described in Examples 1 and 2 clearly demonstrate that the methods and results described in the present invention are operable *in vivo* and that one of ordinary skill would have been able to make and use the claimed invention at the time the invention was made. The applicants respectfully submit that the replacement

Although the vectors chosen for the present exemplification are adenoviral vectors, the applicants submit that the additional data clearly demonstrates that any viral vector capable of transducing a monocyte/macrophage may be used. These vectors include an adeno-associated viral (AAV) vector, a herpes-virus vector or a lentiviral vector (see page 14, lines 5-22 of the application as filed). These vectors do not include a retroviral vector, as disclosed in Ratcliffe *et al* because a retroviral vector cannot transduce a non-dividing cell, such as a monocyte/macrophage.

The applicants also submit that the "sustained expression" concerns raised by the Examiner in relation to the Verma citation are not relevant to the present invention because there is a continual turnover of monocytes/macrophages in the blood stream and at diseased tissue sites. Thus, the continual infiltration of monocytes/macrophages from the blood stream provides a sustained expression of the gene of interest *via* a short term expression from each transduced/transfected cell.

As further evidence in support of the presently claimed invention, the Examiner is requested to consider the following:

It is well known in the art that hypoxia is a powerful regulator of gene expression in a wide range of different cell types (Wang and Sememnza 1993 Proc Natl Acad Sci USA 90:4304) and acts by the induction of the activity of hypoxia-inducible transcription factors such as hypoxia inducible factor-1 (HIF-1), **which bind to cognate DNA recognition sites, the hypoxia-response elements (HREs)** on various gene

"The enhancer may contain elements for regulated expression such as a hypoxia regulated enhancer (for example a binding element for the transcription factor HIF1) or elements which respond to stress or low g/ucose"

and it teaches on page 25, lines 22-24 that:

"Sequences from a region approx. 300-375 bp upstream of the transcription start of the human Enolase A gene were chosen containing three HIF-1 consensus binding sites (Semenza et al 1996 J. Biol. Chem. 271: 32529-32537"

The applicants have demonstrated in the attached Figures 4, 5 and 6 that macrophages express HIF-1α when exposed to hypoxia *in vitro* or in avascular areas of human tumours (Figure 4), human wounds (Figure 5) and human arthritic joints (Figure 6).

In this respect, Figure 4 shows that Macrophages express HIF-1α when exposed to hypoxia in vitro or in avascular areas of human tumours. Immunolocalization of HIF-1α in human monocyte-derived macrophages (A,B) following exposure to either normoxia (20.9% 0_2; A) or hypoxia (0.5% 0_2 ; B) for 16 hours *in vitro*. Immunoreactive HIF-1α is seen in both the cytoplasm and nuclei (arrows) following hypoxic induction. Expression of immunoreactive HIF-1α by CD68-positive macrophages in a human ovarian (C,D) and breast (E,F) carcinoma. Sequential 3μm sections of wax-embedded tumours were immunostained for the pan-macrophage marker, CD68 (C,E) and for HIF-1α (D,F). HIF-1α is present in both the cytoplasms and nuclei of macrophages in these

Figure 5 shows that macrophages express HIF-1α in human wounds

Immunolocalization of HIF-1α by macrophages in a human dermal wound, two weeks post-injury (A,B) and an ovarian carcinoma (C,D). Macrophages (A,C) are highlighted in red using a monoclonal anti-human CD68 coupled to an alkaline phosphatase/Fast Red staining method, and HIF-1 (B,D) in brown using a monoclonal anti-human HIF-1α coupled to a peroxidase/DAB staining method. Scale bars = 50 μm.

Figure 6 shows that macrophages express HIF-1α in human arthritic joints. Immunohistochemical localization of CD68-positive macrophages (brown colour reaction using a peroxidase substrate, top left), HIF-lα (red colour reaction using an alkaline phosphatase substrate, top right) and CD31-positive microvessels (brown, bottom left) in the hypoxic, outer intimal layers of human synovial tissue from a joint with rheuamtoid arthritis.

Summary

Figures 4-6 therefore provide additional evidence that hypoxic conditions can induce the activity of hypoxiainducible transcription factors such as hypoxia inducible factor-1 **(HIF-1)** (Wang and Semenmza 1993 *ibid),* which is capable of binding to cognate DNA recognition sites. the hypoxia-response elements (HREs) and upregulating the expression of a gene associated with the HRE.

The claims are submitted to be supported by an enabling disclosure.

With specific regard to the Examiner's criticism of claim 39, the applicants note that although a constitutive CMV promoter was used in the above murine studies. it

sequence (see page 12, lines 2-18). The regulation of expression of a gene through the manipulation of the promoter sequence is well known in the art (See for example, page 12, lines 2-18.). Likewise, the coupling of hypoxically inducible expression control sequences (promoter) for EPO and PGK genes have been coupled to gene sequences of choice (see page 37, lines 8-11). Thus, these manipulations were well known to the person ordinarily skilled in the art and are enabled by the present specification as well as the generally advanced level of skill in the art.

The Section 112, first paragraph, rejection of claims 42-44 noted in paragraph 4 on page 4 of the Office Action dated January 3, 2001, will be moot upon entry of the above. Similarly, the Section 112, second paragraph, rejection of claim 44 stated in paragraph 5 on page 4 of the Office Action dated January 3, 2001, will be moot upon entry of the above. The applicants note in this regard that the subject matter of claim 42 has been incorporated in the new claim 51. Moreover, canceled claims 43 and 44 (which have been rewritten as new claims 66 and 67) are clearly enabled by the additional data provided as they demonstrate that a composition comprising: a hypoxia and/or ischaemic and/or stress regulatable agent; a mononuclear phagocyte that has coupled thereto, or internalised therein, the hypoxia and/or ischaemia and/or stress regulatable agent; and a binding agent that binds to a cell surface element of the mononuclear phagocyte wherein said binding agent is an adenoviral vector can localise and express a reporter gene at a target site. Figure 2(B) (x 60) of the additional data

would have been routine for the ordinarily skilled person to replace the reporter gene in the Ad. HRE.LacZ in the construct used in Figure 2(B) with, for example, a gene which is cytotoxic for macophages such as a suicide gene (see page 12, lines 19-23).

Finally, claim 44 has been rewritten as new claim 67, to provide the active recitation of method steps requested by the Examiner.

The Section 103 rejection of claims 25-50 over Ratcliffe (U.S. Patent No. 5,942,434) in view of Leek (Cancer Research, Vol. 56, 15 October 1996, pages 4626-4629) and Ferkol (U.S. Patent No. 5,972,900) will be moot upon entry of the above. The amended claims are submitted to be patentable over the cited art and the Examiner's consideration of the following in this regard is requested.

The applicants submit that Ratcliffe *et al* teaches nucleic acid constructs comprising at least one gene encoding a species having activity against disease, operatively linked to a hypoxically inducible expression control sequence. Ratcliffe *et al* provide teachings relating to cancer cell line, fibroblast cell lines and tumour cells transfected with a construct which were implanted under the skin of a mouse. Thus, Ratcliffe *et al* teaches that tumour cells and/or cell lines can be modified under *in vitro* conditions. Ratcliffe *et al* is silent with respect to modifying/manipulating cells other than tumour cells, such as macrophage cells, with a hypoxic construct. Moreover, Ratcliffe *et al* is silent with respect to modifying monocyte/macrophage cells with a viral vector comprising a hypoxic construct, which is capable of transducing such cells, or any

not have been motivated to modify the teachings in Ratcliffe *et al* to make the presently claimed invention.

The applicants submit that Ferkol addresses a problem which is different from the present invention as Ferkol relates to an approach to the treatment of storage diseases that affect the reticuloendothelial system using the macrophage as

"a *primary in vivo target for genetic correction*"

In addition, the teachings in Ferkol *et al* are directed to a non-viral method for introducing genes into a macrophage cell using receptor-mediated gene transter.

Ferkol *et al* do not disclose or suggest either the modification/manipulation of monocytes/macrophages with a viral vector comprising a hypoxic construct, which is capable of transducing such cells, or any advantage/beneficial effects that might be associated with such modifications. In fact, the applicants believe the teachings in Ferkol *et al* would have discouraged the ordinarily skilled person from combining the teachings in Ferkol *et al* with the teachings in Ratcliffe *et al* because Ferkol *et al* state in col 31, lines *51-55* that:

"practical questions regarding the efficiency and specificity of gene delivery using this system (i.e., gene transfer via the mannose receptor) need to be addressed"

Thus, the applicants submit that any attempt to combine the teachings in Ferkol *et al* with Ratcliffe *et al* would run contrary to the teachings in Ratcliffe *et al* because Ferkol *et al* report that expression of a reporter gene can be detected in the liver and

where there is an abundance of tissue macrophages because the mannose receptor fused to the reporter complex mediates gene transfer into macrophages. Thus, even if the method of gene delivery in Ferkol *et al* were successful, it would not have persuaded the ordinarily skilled person to prepare the composition of the presently claimed invention, for delivery of a gene of interest, such as a therapeutic gene, to a hypoxic site which may not be a site such as liver or spleen which has an abundance of tissue macrophages.

Finally, the applicants submit that Leek *et al* relates to the use of the macrophage as a target immuno-inhibition therapy in breast cancer (see last line of the abstract on page 4625). In this regard, Leek *et al* suggest that tumour associated macrophages (TAMs) could be an effective target using agents such as IL-10 or Linomide which block macrophage infiltration. Thus, the Leek *et al* citation provides teachings on how to control macrophage migration, rather than enhance macrophage migration, to a target site, such as a tumour site, in order to deliver a gene to selectively destroy either a tumour cell and/or a monocytes/macrophages at a target tumour site.

It is clear from Leek *et al* that there would have been no motivation for the ordinarily skilled person to look to this teaching to remedy the deficiencies in either Ratcliffe *et al* or Ferkol *et al* because Ratcliffe *et al* and Leek *et al* relate to the selective expression of a gene from a tumour cell. In contradistinction, the presently claimed invention relates to the selective expression of a hypoxic and/or stress and/or ischaemic

In summary, any attempt to combine the teachings in Leek *et al* with the teachings in Ratcliffe *et al* would not have led the ordinarily skilled person to make the compositions or methods of the present invention. Thus, the claimed invention was not obvious over the combination of Ratcliffe *et al*, Ferkol *et al* and Leek *et al*.

Entry of the above amendments is requested. In view of the above and attached, the claims, as amended, are submitted to be in condition for allowance and a Notice to that affect is requested.

Respectfully submitted,

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By: _____

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